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Cytomegalovirus and polyomavirus BK posttransplant

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Abstract

Virus replication and progression to disease in transplant patients is determined by patient-, graft- and virus-specific factors. This complex interaction is modulated by the net state of immunosuppression and its impact on virus-specific cellular immunity. Due to the increasing potency of immunosuppressive regimens, graft rejections have decreased, but susceptibility to infections has increased. Therefore, cytomegalovirus (CMV) remains the most important viral pathogen posttransplant despite availability of effective antiviral drugs and validated strategies for prophylactic, preemptive and therapeutic intervention. CMV replication can affect almost every organ system, with frequent recurrences and increasing rates of antiviral resistance. Together with indirect long-term effects, CMV significantly reduces graft and patient survival after solid organ and hematopoietic stem cell transplantation. The human polyomavirus called BK virus (BKV), on the other hand, only recently surfaced as pathogen with organ tropism largely limited to the reno-urinary tract, manifesting as polyomavirus-associated nephropathy in kidney transplant and hemorrhagic cystitis in hematopoetic stem cell transplant patients. No licensed anti-polyoma viral drugs are available, and treatment relies mainly on improving immune functions to regain control over BKV replication. In this review, we discuss diagnostic and therapeutic aspects of CMV and BKV replication and disease posttransplantation.

Keywords: cytomegalovirus; BK virus; prophylaxis; resistance; T-cells; transplantation; viral infections

Introduction

The key challenge after transplantation is the recognition of alloantigens by immune effectors. The resulting acute and chronic immune reactions cause transient and lasting damage with decreasing organ function and graft loss. In recent years, potent immunosuppressive protocols significantly improved graft survival in solid organ transplantation (SOT) by reducing rejections, across HLA mismatches [1]. However, as illustrated by registry data of 7500 pediatric kidney transplant patients, decreasing hospitalization rates in the first 2 years posttransplant for acute rejection from >30% in 1982 to ~12% in 2002 were paralleled by increasing hospitalization rates for infections from 20.4% to 30.8% [2]. Similarly, infection rates increased in adult kidney transplant recipients of >50 years from 48% to 69% during the first year post-transplantation [3]. In hematopoietic stem cell transplantation (HSCT), summary data from the European Bone Marrow Transplantation on 14 403 HLA-identical siblings with early leukemia indicated a declining mortality due to infections within the first 12 months between 1980 and 2002 from 6% to 1% which in part reflected reduced toxicity of induction and conditioning protocols [4]. However, virus attributed mortality largely persisted, with older age and T-cell depletion as significant risk factors [4].

Virus replication and disease posttransplant results from complex interactions of patient, graft and virus determinants (Figure 1) which are modulated by the net state of immunosuppression [5,6]. Transplant patients are at high risk for acute, typically respiratory viruses transmitted according to their activity in the community. By contrast, viruses persisting in patients or in transplants reactivate in an almost time table-like sequence of first Herpes simplex, then cytomegalovirus (CMV), and varicella-zoster virus [5]. Herpes simplex and varicella-zoster virus are conveniently suppressed by well tolerated drugs like acyclovir and famciclovir peri- and post-transplantation. For CMV, markers of virus-specific cellular immune functions are considered

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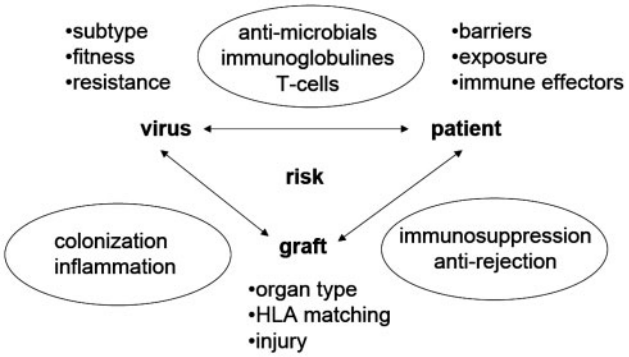

Fig. 1. Interaction of virus, patient and graft (adapted from [131]).

for risk stratification [6]. Thus, CMV seronegative recipients (R−) receiving solid organ transplants from CMV infected, seropositive donors (D+) are at highest risk for CMV replication and disease. The risk also increases in CMV (R+) patients treated with T-cell and/or B-cell depleting antibody regimens administered for induction or rejection. Conversely, CMV (D−/R+) HSCT patients are at higher risk since specific immune effectors depleted by the induction and conditioning regimens cannot be adequately restored by the donor graft, particularly in the presence of graft-versus-host-disease (GvHD) prophylaxis or its treatment. The identification of patients at high risk for CMV replication according to the serostatus of donor and recipient provides the rationale for prophylactic and preemptive administration of antivirals and significantly reduces CMV disease and its associated mortality. However, late manifestations and indirect effects seem to persist as significant challenges. Similar approaches are still being explored for BKV, but, some progress has been made and will be discussed here.

Cytomegalovirus

Virological aspects

CMV belongs to the human herpes viruses and has a linear double-stranded DNA genome of about 235 000 base pairs with more than 200 open reading frames, coding for at least 59 proteins [7,8]. CMV latency and replication is tightly regulated with coordinated expression of immediate-early (IE), early and late genes. IE proteins, e.g. pp72 and IE2 are central regulators of viral gene expression. Early gene proteins like UL97 phosphokinase and UL54 DNA polymerase facilitate viral genome replication, while late proteins e.g. pp65 and glycoprotein B (gB) include structural proteins found in the viral capsid, matrix and envelope. CMV is transmitted via saliva, body fluids, cells and tissues. The seroprevalence depends on socioeconomic status and ranges from 30%–70% in Western Europe and North America [9]. Following primary CMV replication in seronegative individuals, CMV establishes non-replicative infection (latency) in CD34+ myeloid progenitor cells as a major site [10]. Secondary CMV replication in seropositive individuals can be viewed as the net result of activating stimuli and inhibitory immune functions acting at the respective cells and tissues (Figure 1). Activation may result from stress, drugs (catecholamines), inflammatory mediators (TNFα) and hypoxia (oxygen radicals) as encountered during sepsis or ischemia/reperfusion posttransplant and increases CMV IE transcription via NF-kB, AP1 or CREB [11,12]. The state of CMV-specific immune controls together with local microenvironment determines progression to organ-invasive disease in intestines (40%), liver (20%), lungs (10%), kidneys (5%), eyes (1%) and the central nervous system (1%) (Table 1). In addition CMV uses diverse immune evasion mechanisms such as downregulating major histocompatibility complex (MHC) class I molecules, inhibiting NK cells (like gpUL18 [13] or gpUL40 [14]), and producing cytokine homologues like the viral IL10 [15].

Immunological aspects

Neutralizing antibodies predominantly target the glycoprotein B (gB) localized in the viral envelope. In pregnant women with primary CMV infection administration of CMV hyper-immune IgG may reduce CMV disease in infants [16]. In transplant patients, administration of CMV hyper-immune IgG is

Table 1. Effects of Cytomegalovirus and Polyomavirus BK Posttransplant

Direct effects	Indirect effects	Drug effects
Cytomegalovirus		
CMV Disease Syndrome Myelosuppression Hepatitis Colitis Pneumonitis Encephalitis Retinitis	Acute rejection Graft-versus-Host Disease Bronchiolitis obliterans Vanishing bile duct Graft nephropathy Graft vasculopathy Immunosuppression Other infections (Fungal Bacterial, Viral) Post transplant lympho-proliferative disease (PTLD)	Ganciclovir Neutropenia Infections Teratogenicity Foscavir Renal Failure Cidofovir Renal failure Leucopenia Teratogenicity
Polyomavirus BK Polyomavirus associated nephropathy (PVAN) Hemorrhagic cystitis (Renourinary cancer?) (Progressive multi-focal leucencephalopathy-like?)	Acute rejection? Chronic allograft nephropathy? Diabetes? PTLD?	Cidofovir Renal failure Leucopenia Teratogenicity Leflunomide Infections Neutropenia Liver toxicity Quinolones ? yeast infections resistance

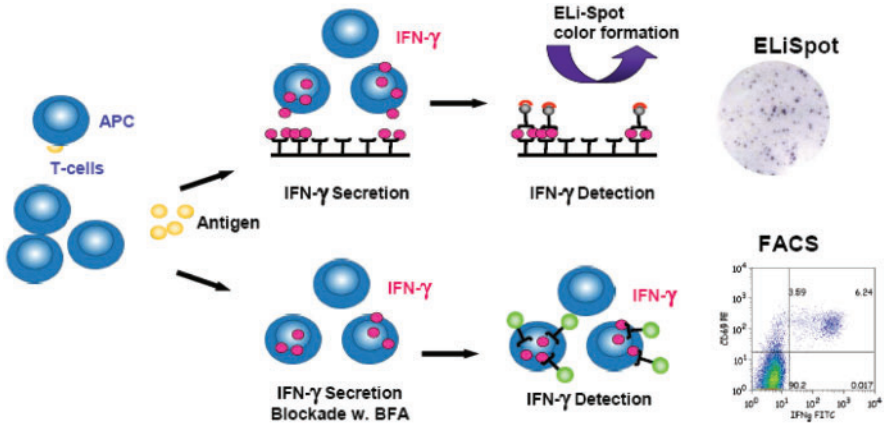

Fig. 2. Quantification of virus-specific interferon-γ (IFN-γ) producting T-cells. Production of INF-γ and/or other cytokines after stimulation with viral epitopes is detected by ELISPOT assay (top) or intracellular cytokine staining (bottom) and has been used as a surrogate marker of CMV-specific T-cell function. BFA: Brefeldin A.

restricted to cases with CMV pneumonitis and antiviral resistant CMV disease. Cellular immunity is central in containing CMV replication as evidenced by increased reactivation in CMV seropositive (R+) transplant recipients treated with T-cell depleting agents. Natural killer cells (NK; CD3-CD56+ CD16+) have a role in early innate defense, but seem to confer only transient protection [17,18]. Disseminated, life-threatening CMV replication has been reported in rare cases with absolute NK cell deficiency [19–21]. In HSCT patients, NK cells are among the first lymphocyte populations to recover. No significant differences in NK cells was found in patients with or without active CMV replication [22]. In renal transplant patients (n = 61) with active CMV replication (78%), no discernible changes in NK cells were reported [23]. Thus, the role of specific and functional T-cells is emphasized for relevant immune containment of CMV.

CMV-specific CD4+ T helper cells and CD8+ cytotoxic T-cells contribute to controlling CMV replication, and protecting against disease [24–26]. Since cytotoxic activities are difficult measure in clinically relevant routine settings, flow-cytometry for MHC-I tetramers painting, intracellular cytokine production or cytokine secretion or ELISPOT assays are commonly used (Figure 2). [27–29]. Interestingly, the range of CMV peptide epitope recognized by CD8 or CD4 T-cells seems rather small and hierarchical. CMV-specific CD8 T cells recognize in up to 40% pp65 (late tegument protein) and IE1 pp72, whereas the remaining activities target pp50, gB, and IE-2pp [30–32]. Additionally, different viral epitopes of one given protein are preferentially recognized by different HLA class I alleles [11,33]. While CMV-specific CD8 T-cells confer immediate control by killing of CMV-replicating host cells, CD4 T-cells seem to be more important for mounting and maintaining longer term antiviral control. Decreasing CMV-reactive CD4 T-cell frequencies during the first months after transplantation correlated with increasing CMV load [34]. CMV pp65-directed responses are more frequently detected [35], but appear later than responses to gB or pp72 (A. Egli and H. H. Hirsch, submitted). Recent data correlated with increasing concentrations of calcineurin-inhibitors (cyclosporine >100 ug/mL; tacrolimus >6 ng/ml) with reduced interferon-γ (IFN-γ) production of T cells. Interestingly, CD8 T-cells seemed more sensitive than CD4 T-cells [25]. Our data in CMV-seropositive kidney transplant patients indicate significantly lower IFN-γ responses in CD4 and CD8 T-cells compared to healthy non-immunosuppressed individuals and even lower levels in kidney transplant patients with ongoing CMV replication [36].

Current management strategies

Antiviral strategies aim at eliminating or reducing CMV replication before CMV disease develops (Figure 3) [5]. CMV disease is stringently defined by the need to demonstrate organ invasiveness by histology [37]. Although developed for research purposes, the definitions also proved helpful in clinical practice for decisions regarding diagnostic procedures and for starting antivirals (Figure 3). Without intervention, the majority of CMV replication and disease occurs early during the first 3 months post-transplantation at the time of the highest immunosuppressive load [38].

Universal prophylaxis with valganciclovir (VGCV) or oral ganciclovir (GCV) for 3 months is now the preferred strategy for high-risk CMV D+/R− SOT recipients in many transplant centers. After discontinuing prophylaxis, still significant rates of CMV replication and disease have been noted which are more difficult to be identified in the outpatient situation [39,40]. In liver transplant recipients, CMV disease after discontinuing prophylaxis was associated with an increased mortality rate [41]. Liver transplant recipients who received antiviral CMV prophylaxis, developed in 8.5% CMV disease at a median of 4.5 months. The mortality was 12% and in 49%

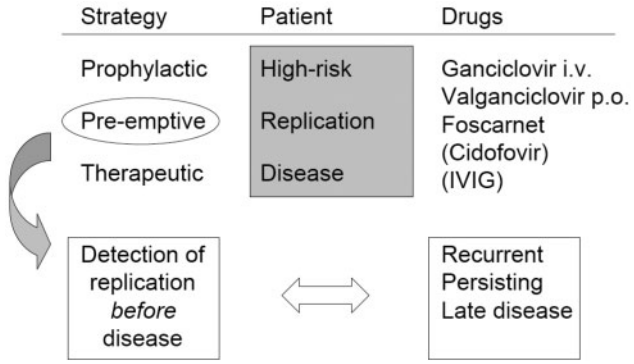

Fig. 3. Antiviral strategies: prophylaxis, pre-emptive treatment and therapy.

associated with CMV replication [42]. Therefore, extending prophylaxis to 6 months posttransplant for CMV (D+/R−) SOT is currently evaluated. Of note, 2% of CMVD+/R− of VGCV treated patients had detectable CMV replication compared to 10% in the oral GCV group. The difference might be attributed to the better oral bioavailability and higher GCV levels in patients on VGCV [43,44]. Since GCV-resistant late CMV disease has been observed under oral GCV in CMV D+/R− SOT [45], a decreased risk of late disease and GCV resistance should be expected [46,47]. This conclusion has to stand the test of clinical routine, where this proportion is likely to be larger than inside of studies.

Preemptive therapy is favored in many centers for CMV R+ SOT recipients, since CMV replication and disease is less likely due to some degree of protection by CMV-specific immunity. This strategy is challenged by the need for sensitive and specific screening procedures operating under clinical routine conditions and by the potential negative impact of indirect effects [48,49]. Quantitative assays detecting CMV in the peripheral blood are generally used such as CMV pp65 antigenemia in buffy coat cells or real-time PCR on plasma or whole blood. Both types of assays show high positive and negative predictive values above 80–90% for CMV disease when combined with thresholds, yet allowing a sufficient time window sufficient to institute antiviral therapy before disease manifestation (Figure 3) [50]. Quantitative PCR has a higher sensitivity especially in HSCT patients and can provide important viral kinetic information. Severely immunosuppressed transplant recipients may show faster CMV dynamics, delayed clearance and more recurrences [51] [7A]. In a recent randomized controlled trial of kidney transplant recipients, prophylaxis reduced significantly CMV replication over pre-emptive treatment (6% *vs* 59%) during the first 100 days [40]. No differences in mean peak CMV load levels or in the time needed to clear the first episode of CMV viremia were noted between the both study arms. However, significantly prolonged viremia was seen in CMV D+/R− patients indicating that transplant patients benefit from (residual) specific antiviral immunity [40]. This patient population is at significant risk of selecting GCV-resistance.

In HSCT patients, prophylaxis is not widely used because of potential myelosuppressive effects of GCV, which may change with new drugs like maribavir. However, pre-emptive therapy has lead to significant reduction of CMV disease during the first 3 months after transplantation (20–30% to <5%) [52]. Nevertheless, a significant survival disadvantage remains for CMV R+ compared to D−/R− HSCT patients, despite the availability of antiviral drugs, sensitive and specific monitoring tools and an overall reduction of early CMV disease. After a median of 169 days, 17.8% had CMV late disease with a mortality of 46%. This corresponds to approximately 10% of all HSCT patients [53].

The prevention of indirect CMV effects is difficult to judge and is likely to be more pronounced in patients with more extensive CMV replication and CMV disease. However, part of the indirect effects could be mediated by CMV-induced immune pathology which may persist beyond actual CMV replication (Table 1). Therefore, an important question is whether treatment of CMV replication with effective antivirals should be accompanied by reducing immunosuppression, a strategy retained from the pre-antiviral era. We believe that the first episode of CMV replication should be treated with sufficient dosing of antivirals, without modifying maintenance immunosuppression. In cases of recurrence, antiviral treatment activating stimuli should be controlled and combined with moderately reduced immunosuppression since CMV-specific immunity might be inadequate. The viral factors causing indirect effects are not well understood and may involve cytokine activation, immunomodulatory effects as well as triggering of alloimmune responses with slowly progressive inflammation with collateral damage in host and graft tissues [54]. In addition, CMV replication may add to the net state of immunosuppression and thus give rise to more fungal infections and PTLD [55]. In kidney transplant patients without CMV prophylaxis, D+/R− patients had shorter graft survival over three years (74%) in comparison to D−/R− patients (82%) [56]. Brennan and coworkers found that HLA-DR mismatching was associated with reduced renal allograft survival after CMV disease [48]. In a classic paper, Lowance et al reported that high-dose valaciclovir prophylaxis reduced not only CMV disease, but also the number acute rejection episodes in CMV D+/R− kidney transplant recipients [57]. A meta-analysis by Small *et al*. showed no significant difference in rejection episodes between the two intervention strategies [58], but there were insufficient data to evaluate graft loss and opportunistic infections.

GCV-resistant CMV replication

In recent years, GCV resistant CMV mutants emerged as a significant problem in transplant patients.

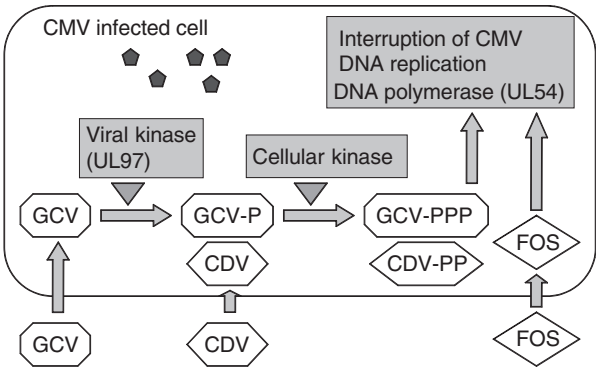

Fig. 4. Mechanisms of UL97 and UL54 associated Ganciclovir resistance. Phosphokinase (UL97) mutations decrease the efficacy of GCV, whereas mutations in the DNA polymerase (UL54) reduce efficacy of all antiviral agents.

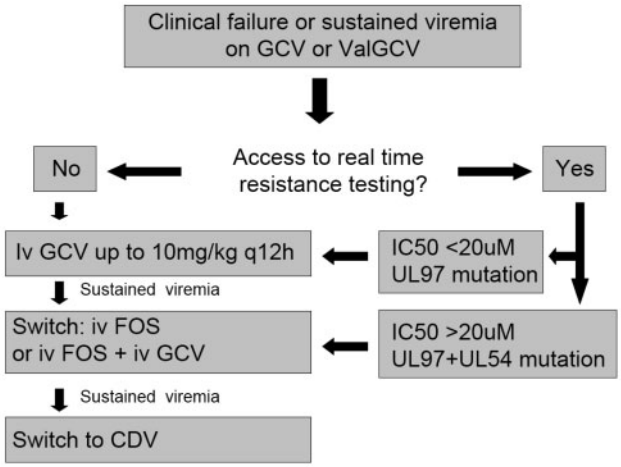

Fig. 5. Recommendations: management of GCV resistant CMV replication (adapted from Preiksaitis *et al.* 2005 AJT 5: 218). GCV ganciclovir, VGCV valganciclovir, FOS Foscarnet, and CDV cidofovir. IC50: concentration with 50% inhibition of viral replication. CMV load testing is recommended weekly during therapy until negative.

Principle risk factors for CMV resistance are insufficient drug levels, frequent recurrence, repetitive treatment, and residual replication, all of which are more likely in patients with impaired or absent CMV-specific immune effectors. Depending on the laboratory method used, GCV resistance is defined as IC50 over 6-12 uM or a two- to five-fold increase in IC50 for viruses during treatment compared to pre-treatment state [59]. Mutations in the viral kinases (UL97 gene) increase the IC50 by reducing the phosphorylation rate of GCV which is required for activation and inhibition of efficient viral DNA replication (Figure 4) [60]. Cidofovir and foscarnet enter the nucleotide pool downstream of UL97 and remain effective alternatives for GCV resistance. Mutations in the viral DNA polymerase (UL54) are less common, but may cause some cross-resistance. In HSCT patients, CMV resistance does not yet seem to be a major issue, probably due to the fact that prophylaxis was rarely used, and treatment was so far administered intravenously. In a European multicenter study only 2 patients had phenotypically confirmed resistance, but 23 clinically were suspected [61]. In SOT, CMV (D+/R−) patients are more prone to develop GCV-resistant CMV replication and disease, particularly when on oral GCV [46]. We predict that the convenient oral administration of VGCV for outpatient treatment will result in more GCV-resistant cases because of suboptimal dosing adjustments and compliance issues.

Current recommendations for management of GCV resistant CMV replication are summarized in Figure 5. If access to phenotypic or genotypic resistance testing is not available in clinically relevant time, intravenous GCV dosage should be increased and/or, if not tolerated (myelosuppression) or viremia persists, switching to foscarnet and to cidofovir should be considered [62]. In cases of clinical or genotypic or phenotypic resistance, reducing immunosuppression and administration of CMV hyper-immunoglobulins should be considered.

Polyomavirus BK

Virological aspects

BKV is closely related to the other human polyomavirus type 2 (JC virus) with a >70% homology of the 5.3 kb circular double-stranded DNA genomes. In the last year, two other polyomaviruses, called WU and KI, have been detected in humans with respiratory infections. The polyomavirus genome structure is conserved and encodes only 6 proteins. The regulatory large tumor antigen (LT-ag) and the small t antigen are early gene proteins, while the capsid VP-1, -2 and -3 and the agnoprotein are late gene proteins. Early and late viral gene expression is driven by the non-coding control region (NCCR) which contains also the origin of DNA replication. Rearrangements of the NCCR occur with persisting BKV replication increasing replication capacity [63,64]. Transmission of BKV occurs typically during childhood (median 4-5 years of age) [65] via oral and respiratory routes, but data suggesting transmission via cells and tissues, in particular by kidney transplantation have been reported [66,67]. Seroprevalence increases to >80% in adults [65]. After primary replication in seronegative individuals, BKV establishes non-replicative infection in the renourinary tract, without known complications for the immunocompetent host. About 5% of healthy individuals intermittently reactivate BKV replication with detectable viruria [68].

Polyomavirus associated nephropathy (PVAN) and late-onset hemorrhagic cystitis are major complications linked to high-level BKV replication in kidney transplant recipients and HSCT patients, respectively [69]. BKV dynamic studies after surgical removal of PVAN-containing allografts revealed a rapid drop of plasma BKV loads. This suggests that the vast

majority or all of the BKV loads in plasma are derived from BKV replication in the allograft. Calculated plasma viral half-life of 1–2 h imply viral turnover of more than 99% per day and a tubular epithelial cell loss of about 10e6 cells per day [70,71].

BKV-associated hemorrhagic cystitis is of late onset approximately more than 10 days after HSCT, as opposed to early onset hemorrhagic cystitis which occurs at the time of conditioning as a toxic side effect to busulfan and irradiation. Late-onset hemorrhagic cystitis occurs typically at the time of engraftment and associated with persistent high-level BKV viruria [72,73]. Therefore, BKV-associated late-onset HC may represent an immune reconstitution disease [74]. Interestingly, BKV is frequently detectable in plasma samples of patients with or developing HC suggesting that local inflammation may increase access to the blood [75]. It is important to note that only about half of HSCT patients with high-level BKV viruria develop hemorrhagic cystitis and that other viral infections, including CMV and adenovirus, may cause similar clinical presentation and may even coexist [76].

Immunological aspects

Neutralizing antibodies target the major capsid protein VP1 and closely correlate with antibody titers measured by type 0 hemagglutination inhibition titers or by BKV VP1-derived virus-like particles [65,77]. These antibodies probably have a role in clearing and protecting from BKV viremia, but might be less effective in case of tissue-invasive disease in transplant patients. In kidney transplant patients, risk factors of BKV replication and disease have been described and include older age, male gender [78], sero-positive donor [79,80], sero-negative recipient [80,81], lack of BKV-specific cellular immune memory compartment [*82*], use of potent immunosuppressive regimens [83–85], HLA C7 negative donor or negative recipients [79], HLA mismatches [86,87] and rejection episodes followed by anti-rejection treatment [86,87]. Most of these factors point to impaired cellular immune functions as a common denominator. The protective effects of BKV antibody titers in this setting is probably not partly related to neutralizing activity [79,86]. More likely, higher antibody titers are measure of recent exposure to BKV with correspondingly larger BKV-specific cellular immune compartment [88]. BKV-specific cellular immunity has been investigated directly measuring IFN-γ responses of PBMC after stimulation with BKV preparations from cell culture, overlapping peptide pools covering the LT-ag and VP1 and observed an increasing activity in patients after PVAN had been cleared following reduced immunosuppression [82,89,90]. Similar results have reported for CD8 T-cells from HLA-0201 kidney transplant patients using labeled MHC-class I tetramers with VP1 derived peptides after short-term amplification cultures [91, 92]. Recent work in our laboratory suggested that VP1 and LT-ag responses were higher in patients with >2 log declining plasma BKV loads. Overall, these responses were more likely to involve CD4 than CD8 T-cells [93,94]. LT directed IFN-γ responses of >69 spot-forming units per 10e6 PBMC in ELISPOT assays identified more patients with >2 log declining plasma BKV loads [95]. If confirmed in prospective studies, combined determination of plasma BKV load and BKV-specific LT-ag-responses might allow distinguishing BKV protected patients from those at high risk for BKV disease progression. Recently, a strong VP1-directed CD8 T-cell response in PBMC was associated with loss of allografts [90], indicating that BKV-specific immune responses might be involved in indirect effects favoring graft failure after kidney transplantation. BK-agno protein albeit significantly expressed during replication of BKV seems not to mount a significant cellular and humeral immune response [96].

In HSCT patients, patients developing BKV viruria and hemorrhagic cystitis are typically seropositive prior to HSCT. Thus, BKV replication is a secondary reactivation following exposure to chemotherapy and irradiation, which also depleted BKV-specific cellular immunity. Early work in HSCT patients found a correlation with increasing antibody titers and BKV viruria [72,97]. The determinants for an immune reconstitution pathology remain to be defined. Further work is needed to better understand the pathogenesis of hemorrhagic cystitis, a prerequisite for better management and intervention.

Current management strategies

Hemorrhagic cystitis complicates HSCT in 5% of patients, between 2–6 weeks post-transplantation. The disease often starts abruptly in hematologically reconstituted patients and may persist for 4–12 weeks, with immobilizing pain and anemic bleeding requiring hospitalization. Treatment remains challenging and currently consists of pain relief, bladder irrigation and in severe cases with direct urologic intervention. Successful treatments with systemic and intravesical cidofovir have been reported [98], but larger studies are lacking. Our own experience with local instillation of cidofovir was negative. Leung et al reported that standard doses of ciprofloxacin may lower BK viruria levels [99]. Some urine BKV loads in patients did not respond to ciprofloxacin and possible resistance was investigated. The clinical impact of ciprofloxacin was difficult to discern as the number of cases was too low to identify a clinical benefit and requires larger prospective studies. In the absence of intervention protocols of proven benefit, there is currently no reason to screen for BKV viruria in the clinical routine setting.

PVAN complicates kidney transplantation in 1–10% of cases, mostly at the end of the first year posttransplantation, with clinically silent, creeping allograft failure in 50–90% (Table 2). Graft loss may occurs in about 50% of cases during the subsequent 6–60 months [100,101]. Persisting BKV

Table 2. PVAN prevalence rates and graft loss

Study	Center	Rates	Graft loss
Mengel *et al.* 2003 [83]	Hannover, Germany	1.1%	71%
Trofe *et al.* 2003 [119]	Cincinnati, USA	2.1%	54%
Buehrig *et al.* 2003 [120]	Rochester NY, USA	2.7%	38%
Ginevri *et al.* 2003 [81]	Genua, Italy	3.0%	33%
Rocha *et al.* 2004 [121]	Durham, NC, USA	3.1%	n.a.
Rahaminov *et al.* 2003 [122]	Petah, Israel	3.8%	14%
Kang *et al.* 2003 [123]	Seoul, South Korea	3.9%	100%
Vasudev *et al.* 2005 [101]	Milwaukee WI, USA	4.0%	48%
Ramos *et al.* 2002 [124]	Baltimore MD, USA	5.1%	82%
Hirsch *et al.* 2002 [86]	Basel, Switzerland	6.0%	0%
Lipshutz *et al.* 2004 [125]	San Francisco, USA	6.0%	56%
Namba *et al.* 2005 [126]	Osaka, Japan	6.9%	33%
Li *et al.* 2002 [127]	Bethesda MD, USA	7.0%	33%
Maiza *et al.* 2002 [128]	Lyon, France	7.1%	50%
Matlosz *et al.* 2004 [129]	Warsaw, Poland	7.9%	n.a.
Moriyama *et al.* 2003 (ASN 2003)	Osaka, Japan	10.3%	22%
Mean		5.0%	46%

Table 3. Prospective Study of Plasma BKV load and Definitive PVAN [130]

Viremia at biopsy	<10e4	10e4–10e5	>10e5	
	$n=21$	$n=23$	$n=31$	
Definitive PVAN	1 (4.8%)	16 (68.4%)	20 (64.6%)	$p<0.001$
Pattern A	1 (4.8%)	8 (34.7%)	4 (13%)	
Pattern B	0	8 (34.7%)	16 (51.6%)	
Pattern C	0	0	0	
S-Crea rise (>20%)	4 (19%)	10 (43.5%)	16 (51.6%)	$p=0.01$

replication is associated with a higher probability of graft loss [102,103] (Table 3):

- PVAN A (early) shows focal virus replication in renal tubular epithelial cells with positive nuclear LT-ag staining, but strong inflammatory infiltrates are lacking (graft loss <10%).
- PVAN B is characterized by extensive interstitial infiltrates and strong cytopathic effects (graft loss ~ 50%).
- PVAN C (late) is dominated by tubulus cell atrophy and interstitial fibrosis, and only few cells with virus replication (graft loss <80%) [104,105].

The definitive diagnosis of PVAN requires allograft biopsies, but is challenged by

1. Focal involvement with false-negative results in 10-30% of cases [102].
2. Acute interstitial rejection which is morphologically and molecularly hardly distinguishable [87,106,107]
3. BKV-specific immune reconstitution, after reducing immunosuppression [100].
4. Chronic allograft nephropathy in late PVAN C [108].

Therefore, testing for BKV replication in the urine has become the most pivotal test to exclude PVAN in 65%-85% of kidney transplant patients, whereas in patients with detectable viruria, plasma BKV loads allowed to diagnose "presumptive PVAN" in cases with confirmed higher values to the equivalent of >10 000 copies/ml [86,102,109–111] (Table 3). Screening for BKV replication is therefore recommended 3 monthly during the first 2 years posttransplant, when allograft biopsies are performed for any reason, or when allograft dysfunction occurs [109].

Reducing immunosuppression currently is considered to be the intervention of choice. Although it is widely accepted that earlier intervention is more likely to preserve allograft function, there are currently three major proposals when to reduce immunosuppression: 1. Treat histological confirmed cases with decreased allograft function ('definitive PVAN', typically pattern B–C) 2. Treat histological confirmed cases with baseline allograft function ('definitive PVAN', typically pattern A>B). 3. Treat patients with persistently high plasma BKV load, but negative or unknown histology result ('presumptive PVAN'). These options have not been fully elucidated or compared to each other regarding efficacy and outcome.

Reported strategies for reducing the immunosuppressive load are:

1. Reduce calcineurin inhibitor trough levels (Tacrolimus ≤6 ng/mL, cyclosporine <125 ug/ml.
2. Reduce antiproliferative agent by 50% (Mycophenolate mofetil ≤1 g per day, Azathioprine ≤75 mg per day in adult patients).
3. Discontinuing components of triple drug therapies (mostly mycophenolate mofetil) [85,112], some replace with leflunomide (>4 μg/ml) or sirolimus (<6 ng/ml).

When simultaneous rejection is suspected, anti-rejection treatment might be given priority. In a second step immunosuppressives can then be reduced [113]. Potential antivirals like cidofovir or leflunomide showed some efficacy in-vitro [114–118], while other

antiviral drugs did not. However larger controlled trials of these agents are still missing.

Conclusion

Significant progress has been made in the definition of CMV and BKV infection, replication and disease. Although both viruses are opportunists in the setting of transplantation, with potential indirect effects, the clinical problems currently associated with either virus are fundamentally different. CMV can affect any organ system, with substantial morbidity and mortality, all of which can be essentially controlled by effective antivirals. BKV on the other hand causes severe pathologies in the renourinary tract in a limited number of kidney transplant and HSCT recipients. The absence of effective anti-polyomaviral drugs renders BKV treatment strategies largely dependent on immunological containment of BKV replication. Access to invasive procedures and biopsy workup is required for definitive diagnosis of CMV and BKV disease. However, for both agents, the most relevant diagnostic study in the clinical setting is early detection and quantification of virus replication in blood. Assays quantifying virus-specific cellular immune responses in real-time are important new avenues to be explored to better predict risk of replication and disease and to optimize clinical management.

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